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Association of an insertion/deletion polymorphism in *IL1A* 3′-UTR with risk for cervical carcinoma in Chinese Han Women

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ABSTRACT

Emerging evidence has demonstrated that polymorphisms of interleukin-1 (IL-1) may be involved in human tumorigenesis by regulating the production of this cytokine. Previous studies have investigated the association between two genetic variants (rs3783553 and rs17561) of *IL1A* and many diseases. The present study was conducted to evaluate whether these two variants are associated with cervical carcinoma (CC). These two polymorphisms were genotyped in 319 CC patients and 424 healthy controls by polymerase chain reaction polyacrylamide gel electrophoresis (PCR-PAGE) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Significantly reduced CC risk was observed to be associated with the insertion allele of rs3783553 ($P = 0.014$, OR = 0.71, 95% CI = 0.57–0.88). Stratification analysis based on different certain clinical features showed that patients with the heterozygous genotype were associated with a reduced predisposition advancing to clinical stage II-III or developing non-squamous cell carcinoma. Furthermore, patients with the insertion homozygous genotype were also associated with a reduced risk to have a poor tumor differentiation. No significant association was observed between rs17561 and CC. The present study provided evidence that the rs3783553 in *IL1A* 3′-UTR is inversely associated with CC risk, suggesting an important role IL-1α may play in cervical carcinogenesis.

1. Introduction

Cervical carcinoma (CC) is the third most common gynecological neoplasm worldwide [1]. The most important etiologic agent in the pathogenesis is human papillomavirus (HPV) [2]. However, the majority of HPV carriers do not develop this disease demonstrating that high-risk human papillomavirus (HPV) persist infection is a necessary but not sufficient factor for the development of CC. To date, it has been generally accepted that cervical carcinoma is multifactorial in origin, and immunological mechanism may play an important role in its pathogenesis. Previous study has suggested that women with reduced pro-inflammatory responses are more susceptible to viral persistence and the consequential development of cervical neoplasia [3]. Cell-mediated immunity (CMI), which has been regarded as a critical component in control of both HPV infection and progression to cancer, is associated with macrophages, NK, and T-cell infiltrates [4]. Presented as the immune mediator, which may be involved in these progressions, cytokine is presumed to play a role in cervical carcinogenesis [5]. Despite extensive researches in this malignancy, our understanding of the pathogenesis of CC is still incomplete.

The interleukin-1 (IL-1) family consists of IL-1alpha (IL-1α), IL-1beta (IL-1β), and IL-1 receptor antagonist (IL-1RA), has been

Abbreviations: IL-1, interleukin-1; IL-1α, interleukin-1alpha; IL-1β, interleukin-1beta; IL-1RA, interleukin-1 receptor antagonist; CC, cervical carcinoma; SCC, squamous cell carcinoma; PCR-PAGE, polymerase chain reaction polyacrylamide gel electrophoresis; 3′-UTR, 3′-untranslated region; HPV, human papillomavirus; I, the insertion allele; D, the deletion allele; CMI, cell-mediated immunity; OR, odds ratio; CI, confidence interval.

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thought to be involved in chronic inflammation and cancer. Subsequently, growing evidence has suggested its implication in tumor proliferation, angiogenesis, tumor invasion, metastases and patterns of interactions between malignant cells and the host's immune system [6–10]. IL-1α, which is involved in numerous immune responses and inflammatory process, is said to play an important role in human carcinogenesis [11,12]. Previous research has identified that production of IL-1α and other inflammatory cytokines induced leukocyte infiltration, and resulted in carcinoma formation [13]. An aberrant expression of IL-1α has been observed in several tumor tissues, such as hepatocellular and nasopharyngeal carcinoma [8,14]. To date, mounting evidence suggested the production of cytokines may be genetically determined, and genetic variations of immune system genes may influence the genetic susceptibility to CC [15–18].

The human genes encoding IL-1α (*IL1A*) and IL-1β (*IL1B*) are located within a 430 kb region on chromosome 2q14.2. Several polymorphisms of *IL1A* and *IL1B* correspond with the alterations of IL1-α and IL1-β protein expression have been implicated in the etiologies of human disorders such as polycystic ovary syndrome and nasopharyngeal carcinoma [19,20]. Existing researches have revealed the association between polymorphisms of *IL1A* and several gynecological diseases, such as endometriosis, polycystic ovary syndrome and ovarian cancer [15,21,22]. The rs3783553, a TTCA insertion/deletion polymorphism located in the 3′-untranslated regions (3′-UTR) of *IL1A*, has been suggested to be associated with risk for hepatocellular carcinoma and nasopharyngeal carcinoma, possibly through regulating the expression of IL-1α levels [8,19,23]. The rs17561, a G/T polymorphism of *IL1A* has been observed to be involved in numerous diseases such as ankylosing spondylitis, endometriosis and ovarian cancer, probably by leading a predicted intolerable amino acid change [12,21,24]. However, the association between these two polymorphisms and CC risk remained unclear. Therefore, a hospital based case-control study was performed to test whether these two polymorphisms in *IL1A* are associated with the susceptibility to CC in Chinese Han women.

2. Materials and methods

2.1. Subjects

The present study was performed with the approval of the ethics committee of the West China Second University Hospital of Sichuan University and all the subjects gave written informed consent to participate. A hospital based case-control study was conducted including 319 unrelated women with CC ranging in age from 25 to 71 years old (mean ± SD, 44.32 ± 8.62) between June 2008 and October 2012 at the Second University Hospital of Sichuan University. The diagnosis of CC was confirmed in all cases by histological examination of tissue from biopsy or resected specimens. Histopathological and clinical data were collected from the hospital record section. A group of control subjects including 424 healthy women ranging in age from 22 to 70 years old (mean ± SD, 42.99 ± 8.48) was selected randomly from a routine health survey in the same hospital. Subjects with any personal or family history of CC or other serious disease were intentionally excluded. All subjects were Chinese Han population living in Sichuan province of southwest China. Medical records were reviewed for patients' characteristics, including age at diagnosis, pathological type, clinical stage, tumor differentiation, lymph node status, and parametrial invasion.

2.2. Genotyping

Genomic DNA of each individual was extracted from 200 μl of EDTA-anticoagulated peripheral blood samples by a DNA isolation kit from Bioteke (Peking, China). The procedure was performed according to instruction manual. The primers used for amplification of the rs3783553 were 5′-ATT GTT CCG ATC TTT GAC TC-3′ and 5′-TGA TAA CAG TGG TCT CAT GG-3′ [8], the primers used for amplification of the rs17561 were 5′-TCT GCA CTT GTG ATC ATG GTT-3′ and 5′-AGC AGC CGT GAG GTA CTG AT-3′. The PCR reactions were performed in a total volume of 25 μl, including 2.5 μl 10× PCR buffer, 1.5 mmol/L $MgCl_2$, 0.15 mmol/L dNTPs, 0.5 μmol/L each primer, 100 ng of genomic DNA and 1U of Taq DNA polymerase. The PCR conditions for rs3783553 were 94 °C for 4 min, followed by 32 cycles of 30 s at 94 °C, 30 s at 60 °C and 30 s at 72 °C, with a final elongation at 72 °C for 10 min. The PCR conditions for rs17561 were 94 °C for 4 min, followed by 32 cycles of 30 s at 94 °C, 30 s at 63 °C and 30 s at 72 °C, with a final elongation at 72 °C for 10 min. Two microliters of PCR products were separated by a 6% polyacrylamide gel and stained with 1.0 mg/ml argent nitrate. PCR products for rs17561 were digested overnight with HphI and the digested PCR products were separated by a 6% polyacrylamide gel and stained with 1.0 mg/ml argent nitrate: allele T is cuttable, yielding two fragments of 126 and 45 bp, allele G is uncuttable and the fragment is still 171 bp. About 20% of the samples were randomly selected to perform the repeated assays and the results were 100% concordant. The genotypes were confirmed by DNA sequencing analysis.

2.3. Statistical analysis

All data analyses were carried out by SPSS 13.0 statistical software (SPSS Inc, Chicago, IL, USA). Allele and genotype frequencies of these two polymorphisms were obtained by directed counting and Hardy–Weinberg equilibrium was evaluated by the chi-square test. Genotypic association tests in a case-control pattern assuming codominant, dominant, recessive, overdominant, or log-additive genetic models were performed using SNPstats [25]. Odds ratio (OR) and respective 95% confidence intervals (CI) were reported to evaluate the effects of any difference between alleles and genotypes. A $P < 0.05$ was regarded as statistically significant.

3. Results

Both rs3783553 and rs17561 polymorphism were successfully genotyped in 319 CC patients and 424 healthy control subjects. Genotype distribution of these two polymorphisms in cases and control subjects were consistent with the Hardy–Weinberg equilibrium. Allele frequency of these two polymorphisms for 319 patients and 424 healthy control subjects are shown in Tables 1 and 2. Significantly reduced CC risk was observed to be associated with the insertion (I) allele of rs3783553 ($P = 0.014$, OR = 0.71, 95% CI = 0.57–0.88). But no statistically significant association was observed between CC risk and alleles of SNP rs17561 ($P = 0.91$, OR = 1.05, 95% CI = 0.68–1.60).

A significantly reduced risk for CC was found to be associated with the II genotype of rs3783553 in a codominant model, compared with DD (deletion/deletion) genotype ($P = 0.006$, OR = 0.46, 95% CI = 0.28–0.77). Compared with (DD + DI) genotype, II genotype carriers also have a reduced CC risk in a recessive model ($P = 0.01$, OR = 0.54, 95% CI = 0.33–0.87). Moreover, in a dominant model, significantly reduced CC susceptibility was also associated with allele I carriers ($P = 0.009$, OR = 0.68, 95% CI = 0.50–0.91). But for genotypic association analysis, no statistically significantly difference was observed between CC patients and control subjects for rs17561 polymorphism.

To further evaluate whether these two polymorphisms were associated with certain clinical features of patients with CC, we performed stratified analyses for genotype distribution in CC